

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

1-44. (Cancelled).

45. (New) A method for the preparation of MDCK cells for use in the production of at least one virus, the method being discontinuous and comprising:

- a) culturing MDCK cells that are attached to a substrate to form a preproduction batch,
- b) dividing the cells of the preproduction batch into a first part and a second part,
- c) employing said first part for the preparation of at least one production batch for the production of at least one virus, and
- d) employing the second part as a seed for the preparation of at least one subsequent preproduction batch,
- e) employing a first portion of the cells of the at least one subsequent preproduction batch for the preparation of at least one subsequent production batch for the production of at least one virus,

wherein the cells of the at least one production batch in c) have a different passage number than the cells of the at least one subsequent production batch in e), and

wherein the passage number of each production batch is between master cell bank and extended cell bank.

46. (New) The method according to claim 45, wherein the method further comprises culturing the cells of the at least one subsequent preproduction batch in d) to obtain a greater cell population.

47. (New) The method according to claim 45, wherein the method further comprises repeating steps b), c), d), and e), wherein the repeating comprises obtaining a second portion of the cells of the at least one subsequent preproduction batch of d), optionally culturing the second part of the cells to obtain a greater cell population, and using the second part of the cells for the preproduction batch of b).

48. (New) The method according to claim 45, wherein the proportion of cells forming said first part of the preproduction batch in b) ranges from 80% to 90%, and wherein the proportion of the cells forming the second part of the preproduction batch in b) ranges from 10% to 20%.

49. (New) The method according to claim 45, wherein the method further comprises:

- i) transferring the first part of the cells of the preproduction batch to a first bioreactor to be used for the preparation of at least one production batch, and
- ii) transferring the second part of the cells of the preproduction batch to a second bioreactor to be used as a seed for the preparation of at least one subsequent preproduction batch.

50. (New) The method according to Claim 45, wherein the first preproduction batch is prepared from a working seed stock by at least one passage.

51. (New) The method according to Claim 49, wherein the first preproduction batch is prepared from a working seed stock by at least one passage.

52. (New) The method according to Claim 49, wherein the cells are released from said substrate prior to each transfer step.

53. (New) The method according to claim 52, wherein the substrate comprises particulate matter or a solid support.

54. (New) The method according to claim 53, wherein the solid support comprises hollow fibers or micro-carriers or macro-carriers in suspension.

55. (New) The method according to claim 52, wherein the cells are embedded in a carrier.

56. (New) The method according to claim 55, wherein the carrier is a micro-carrier.

57. (New) The method according to claim 52, wherein the cells are released from said substrate with a proteolytic enzyme.

58. (New) The method according to claim 57, wherein the proteolytic enzyme is trypsin.

59. (New) The method according to claim 57, wherein the cells are treated with PBS and/or EDTA prior to exposure to the proteolytic enzyme.

60. (New) The method according to claim 45, wherein the cells are parked at a certain passage number by exposure to an ambient temperature ranging from 17 to 32 degrees C.

61. (New) The method according to claim 60, wherein said parked cells are revitalised to log growth by raising the temperature and changing the culture media.

62. (New) The method according to claim 45, wherein the cells are frozen at a temperature of less than -80 degrees C in bulk, and thawed prior to use.

63. (New) The method according to claim 45, wherein the extended cell bank is validated and fully characterized with respect to growth characteristics, freedom of adventitious, extraneous and endogenous agents at the different stages, karyology, and iso-enzyme analysis.

64. (New) A method for the preparation of MDCK cells for use in the production of at least one virus, said method being discontinuous and comprising:

- a) culturing MDCK cells that are attached to a substrate to form a preproduction batch, and
- b) forming at least one first production batch and at least one second production batch from the cells of the preproduction batch,

wherein the cells of the at least one first production batch have a passage number different from the cells of the at least one second production batch, and

wherein the passage number of each production batch is between master cell bank and extended cell bank.

65. (New) The method according to Claim 64, wherein:

- a) a part of the cells of the preproduction batch are transferred for the preparation of the at least one production batch, and
- b) a part of the cells of the preproduction batch are transferred to be used as a seed for the preparation of the at least one subsequent preproduction batch.

66. (New) The method according to Claim 64, wherein a first preproduction batch is prepared from a working seed stock by at least one passage.

67. (New) The method according to Claim 65, wherein a first preproduction batch is prepared from a working seed stock by at least one passage.

68. (New) The method according to Claim 65, wherein the cells are released from said substrate prior to each transfer step.

69. (New) The method according to Claim 68, wherein the substrate comprises particulate matter or a solid support.

70. (New) The method according to Claim 69, wherein the solid support comprises hollow fibers or micro-carriers or macro-carriers in suspension.

71. (New) The method according to Claim 68, wherein the cells are embedded in a carrier.

72. (New) The method according to Claim 71, wherein the carrier is a micro-carrier.

73. (New) The method according to Claim 68, wherein the cells are released from said substrate with a proteolytic enzyme.

74. (New) The method according to Claim 73, wherein the proteolytic enzyme is trypsin.

75. (New) The method according to Claim 73, wherein the cells are treated with PBS and/or EDTA prior to exposure to the proteolytic enzyme.

76. (New) The method according to claim 64, wherein the extended cell bank is validated and fully characterized with respect to growth characteristics, freedom of

adventitious, extraneous and endogenous agents at the different stages, karyology, and iso-enzyme analysis.